

Functional Ecology

Environmental filtering and convergent evolution determine the ecological specialisation of subterranean spiders

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Author contribution

MI and SM performed fieldwork. AJD measured specimens' traits. SM analysed ecological data with suggestions by PC, and wrote the first draft of the paper. MA conducted the analyses of the molecular data and wrote relative sections. CF provided suggestions on the evolutionary framework and writing of the introduction and discussion. All authors contributed to the writing of the paper through discussions and additions to the text.

Conflict of Interest

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Abstract

1. Ecological specialisation is an important mechanism enhancing species coexistence within a given community. Yet, unravelling the effect of multiple selective evolutionary and ecological factors leading the process of specialisation remains a key challenge in ecology. Subterranean habitats provide highly replicated experimental arenas in which to disentangle the relative contribution of evolutionary history (convergent evolution vs character displacement) and ecological setting (environmental filtering vs competitive exclusion) in driving community assembly.

2. We tested alternative hypotheses about the emergence of ecological specialisation using the radiation of a lineage of sheet-weaver cave-dwelling spiders as model system. We observed that at the local scale, a differential specialisation to cave microhabitats generally parallels moderate levels of morphological similarity and close phylogenetic relatedness among species. Conversely, geographic distance contributed little in explaining microhabitat occupation, possibly mirroring a limited role of competitive exclusion. Yet, compared to non-coexisting species, co-occurring species adapted to different microhabitats showed lower morphological niche overlap (i.e. higher dissimilarity) and deeper genetic distance.

3. The framework here developed suggests that in the subterranean domain, habitat specialisation is primarily driven by environmental filtering, secondarily by convergent evolution, and only marginally by character displacement or competitive exclusion. This

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pattern results in the establishment of replicated communities across geographical space, composed by ecologically equivalent species. Such process of community assembly well explains the numerous adaptive radiations observed in subterranean habitats, an eco-evolutionary pattern well documented in oceanic islands or mountain summit communities.

Keywords: cave, biotic interactions, functional traits, n-dimensional hypervolume, niche space, phenotypic variability, subterranean biology, Western Italian Alps

INTRODUCTION

The structure of local and regional species assemblages is the outcome of multiple eco-evolutionary processes (Vellend, 2010; Weber, Wagner, Best, Harmon, & Matthews, 2017). Ecological specialisation, i.e. a differentiation of community members with respect to microhabitat use, resource exploitation, or both, is the basis of the stable coexistence between species, thereby importantly contributing to community assembly (Chesson, 2000). Ecological specialisation leads to the differentiation of functional traits linked to ecological niche. Abiotic and biotic factors may drive differences in ecological traits (Emerson & Gillespie, 2008), and trigger evolutionary changes (Schluter, 2000). Yet, eco-evolutionary processes operate in such way that similar patterns can emerge through evolutionary selective and evolutionary-neutral ecological processes, masking their relative importance. Thus, quantifying how diverse processes integrate into habitat specialisation remains challenging.

A species colonizing a novel habitat encounters a specific set of environmental conditions. The success of the colonization depends on functional traits underlying its survival therein. Species lacking appropriate traits will fail in colonizing the new environment, a phenomenon often referred to as environmental filtering (Cornwell, Schilck, & Ackerly, 2006; Kraft et al., 2015). The expected outcome is that species thriving in similar habitats should share their niche-related functional traits (Webb, Ackerly, McPeck, & Donoghue, 2002). Additionally, interspecific interactions act hand in hand with habitat filtering. Within the same

habitat, competition can prevent colonization or eventually eliminate one of the species through competitive exclusion (Chesson, 2000; Scheffer & van Nes, 2006). Competition may also prompt character displacement, and segregation of ecologically distinct species into different niches (Chesson, 2000; Schluter, 2000). An expected outcome of interspecific competition is again ecological distinctness of community members thriving in different habitats (Vergnon, Leijts, van Nes, & Scheffer, 2013; Webb et al., 2002). Both environmental filtering and convergent evolution versus competitive exclusion and character displacement inevitably act jointly upon coexisting species, making them similar within habitat and different across habitats (Barabás, D'Andrea, Rael, Meszéna, & Ostling, 2013; Ingram & Shurin, 2009; Kraft, Valencia, & Ackerly, 2008).

Functional traits also evolve in time, adding an additional level of complexity to the process of community assembly. Functional traits may be contingent upon species' ancestors and change little over an evolutionary timescale (Wiens et al., 2010), insofar as trait evolution is constrained by historical events that are often random ("historical contingency"). Clades with such contingent functional traits advantageously colonize specific habitats (Wiens, 2011). Hence, environmental filtering and historical contingency jointly yield phylogenetically related and phenotypically similar species segregated in similar habitats, implicitly hypothesizing that phylogenetic origin approximates species' ecology (Cavender-Bares, Kozak, Fine, & Kembel, 2009). By contrast, some species may evolve specific traits in response to environmental demands in an ecological time (Herrel et al., 2008). In similar situations, closely related species diverge in their response to interspecific competition or habitat shift—or both, and may exhibit character displacement (Martin & Pfennig, 2009; Pfennig & Pfennig, 2009; Schluter, 2000; Stuart & Losos, 2013). In such cases, it can be expected that species segregated in similar habitats comprise phenotypically similar yet phylogenetically unrelated species that attained their similarity independently through convergent evolution (trait convergence; Losos, 2011).

Here, we explore the origin of ecological specialisation of species on a regional scale, in order to quantify the relative contribution of evolutionary history, interspecific competition, and environmental filtering in driving community assembly.

Our model system is a lineage of sheet-weaver spiders in the genus *Troglohyphantes* (Araneae, Linyphiidae), distributed in caves and other subterranean habitats in a well-defined alpine region of the Western Palearctic (Isaia, Mammola, Mazzuca, Arnedo, & Pantini, 2017). We argue here that subterranean species are a particularly informative natural model system in phylogenetic community ecology, for at least three reasons. First, subterranean communities are often composed of species that independently colonized and adapted to the subterranean environment, allowing us to study multiple replicates of the surface-subterranean transition (Derkarabetian, Steinmann, & Hedin, 2010) and thus to assess the role of historical contingency and convergence. Second, subterranean species are subjected to stratified environmental selection. The subterranean environment is generally compartmentalized into a series of nearly distinct microhabitats differing in light availability (Tierney et al., 2017), size of habitat pores (Pipan & Culver, 2017) and connectivity to the surface (Gers, 1998). A mosaic structure of subterranean microhabitats thereby provides a series of distinct experimental settings defined by distinct habitat-filtering properties (Trontelj, Blejec, & Fišer, 2012). Third, subterranean communities are simpler than surface ones (Gibert & Deharveng, 2002), being composed of few species that mainly compete for space (Bourne, 1976; Mammola, Piano, & Isaia, 2016; Resende & Bichuette, 2016) and food resources (MacAvoy, Braciszewski, Teng, & Fong, 2016; Novak et al., 2010). In summary, this is a simple, ecologically stable, and highly replicated setting where the relative contribution of phylogeny, competition, and filtering in driving ecological specialisation should be more easily quantifiable.

The aim of this study is to explore mechanisms behind ecological specialisation, which we achieved by decomposing habitat specialisation of *Troglohyphantes* spiders into evolutionary history, functional space, and geographical distance. We first selected morphological functional traits and explored whether these are related to the environmental conditions of the different subterranean compartments (i.e. microhabitats). Then, by means of null modelling, regression models, and variance partitioning analysis, we assessed the relative contribution of phylogenetic effects and environmental filtering in explaining specialisation across the different microhabitats inhabited by these species. If most of ecological divergence derived through niche conservatism, we would expect

species from the same microhabitat to be phylogenetically and functionally clustered (i.e. trait conservatism). By contrast, if ecological divergence derived mainly from convergent evolution, we expect functional but not phylogenetic clustering (trait convergence). Finally, we tested whether interspecific competition, inferred from spatial data, facilitates phylogenetic or ecological divergence.

MATERIALS & METHODS

Model organism and study area

The largest spider diversification in subterranean habitats in Europe belongs to the genus *Troglohyphantes* (Linyphiidae) (Mammola, Cardoso, Ribera, Pavlek, & Isaia, 2018). This genus includes several closely related lineages that are typically found in caves, mines, soil litter, air-filled voids in rocky debris, and other shaded and moist habitats (Deeleman-Reinhold, 1978; Isaia et al., 2017). We limited our study to the Western Italian Alps, a coherent biogeographic area in which *Troglohyphantes* diversity is particularly well-documented (Isaia, Lana, & Pantini, 2010; Isaia et al., 2017, 2011; Isaia & Pantini, 2010; Mammola, Isaia, & Arnedo, 2015; Mammola, Piano, Malard, Vernon, & Isaia, 2019). This area is inhabited by 15 endemic species, all subterranean adapted, although showing different levels of specialisations and affinities with the subterranean medium (Figure 1a). All *Troglohyphantes* diversity in the region was considered in this study, including two species currently under description that were formerly attributed to *T. vignai*. Note that one of these species (*Troglohyphantes* sp_2) was later excluded from the analyses, given that too few specimens were available for morphological analyses (Table 1). The number of *Troglohyphantes* species for each cave in this area ranges from one to three, with most caves having two species segregated in different microhabitats (Isaia et al., 2017; Mammola, Arnedo, et al., 2018).

Microhabitat classification

Based on morphological features and thermal tolerance preference, Western Alpine *Troglohyphantes* have been recently subdivided into classes of subterranean

adaptation, namely low, intermediate, and highly subterranean adapted species (Mammola et al., 2019). We assumed here that species with different levels of adaptation should be able to preferentially exploit different microhabitats within the subterranean domain (Figure 2). Although morphological adaptation does not necessarily correlate with subterranean habitat occupation, this is most often the case (Desutter-Grandcolas, 1997) and should be true for *Troglohyphantes* in the Western Italian Alps based on available evidence (Mammola et al., 2019). More specifically, low adapted *Troglohyphantes* species primarily inhabit external or cave entrance microhabitats (approx. 0–10 m from the surface), intermediate adapted species shallow cave microhabitats (approx. 5–50 m from the surface), and highly adapted species deep cave microhabitats (typically >50 m from the surface). Additionally, two species—*T. giachinoi* and *T. iulianae*,—despite showing intermediate adaptations, have a significantly smaller body size compared to the other species (Figure 2), enabling them to exploit shallow interstitial habitat with small sized voids (Mammola, Arnedo, et al., 2018). This specific habitat is often referred to as *Milieu Souterrain Superficiel* (MSS)—see Mammola et al. (2016) for a review on the subject. These four microhabitat types (“Cave entrance”, “Shallow cave”, “Deep cave”, and “MSS”) were used as categorical grouping variable in all analyses.

Morphological traits

We examined 9 to 15 female specimens for each species (Table 1) for a number of morphological traits related to body size, trophic specialisation, and degree of subterranean adaptation (Table 2). Males of *Troglohyphantes* are usually less abundant than females, and thus we lacked sufficient sample size for replicating the analyses on both sexes, which limited our ability to assess the potential biased induce by sexual size dimorphism in our analyses (McLean, Garwood, & Brassey, 2018). By considering only one sex, we excluded the confounding effect of potential morphological variation that may not be outcome of subterranean adaptation, but of sexual selection.

We acquired measures using a Leica M80 stereoscopic microscope. We took

measurements in millimetres (mm), from digital pictures made with a Leica EC3 digital camera and calculated with the Leica LAS EZ 3.0 software (Leica Microsystems, Switzerland). To minimize human error, we took three independent measures of each structure and averaged them.

For each leg, we measured the length of the femur, tibia, metatarsus and tarsus. To estimate eye regression, we measured the diameter of anterior lateral eyes (ALE), anterior median eyes (AME), posterior median eye (PME) and posterior lateral eyes (PLE), and the total length of the anterior and posterior eye lines. To estimate overall body size and shape, we measured sternum length and width and cephalothorax height and width. Height of the cephalothorax was measured at the eye region, starting from the clypeus base to the top of the profile. In addition, we measured the length of the chelicerae and fang, and the presence or absence of pigment in the cuticle of the spider abdomen (eye estimated). From these morphological measures we derived functional traits with specific adaptive meanings, as detailed in Table 2.

Molecular phylogeny

We gathered DNA sequence data for 38 *Troglohyphantes* species including both Western alpine representatives and species from additional regions spanning most of the currently known distribution of the genus (Supplementary Material Appendix S1). We sequenced fragments of four genes: the mitochondrial genes encoding cytochrome c oxidase subunit I (COI), 16S ribosomal RNA (16S), and the nuclear genes for histone H3 (H3) and 28S ribosomal RNA (28S). Most COI sequences were available in public repositories from previous studies (Isaia et al., 2017; Mammola, Arnedo, et al., 2018). Additional sequences were obtained following the protocols detailed in Arnedo et al. (2009) and Mammola *et al.* (2015).

We edited and managed sequences using Geneious v. R10.2.6 (Kearse et al., 2012). We aligned ribosomal sequences using the online version of the program MAFFT v.7 (Katoh, Asimenos, & Toh, 2009), implementing the G-INS-i strategy. We inferred the maximum likelihood tree of the concatenated data matrix (~2.5 Kb) with IQ-TREE v. 1.6.11 (Nguyen, Schmidt, Von Haeseler, & Minh, 2015). We used IQ-

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TREE to first select the best-fit partitioning scheme and corresponding evolutionary models (Kalyaanamoorthy, Minh, Wong, Von Haeseler, & Jermiin, 2017), and then to infer the best tree and estimate clade support by means of 1000 replicates of non-parametric bootstrapping. We conducted Bayesian (BI) analysis and divergence time estimation in BEAST v. 1.8.4 (Drummond, Suchard, Xie, & Rambaut, 2012). We defined partitions and models following results of the ModelFinder analysis in IQ-TREE. We specified a birth and death tree prior and an unlinked relaxed uncorrelated lognormal clock for each gene. Because of the lack of fossil record for the genus, we relied on spider substitution rate estimates available in the literature for estimating absolute divergence times (Bidegaray-Batista & Arnedo, 2011). We assigned a normal distribution on the substitution rate prior (ucdl.mean), truncated at 0, with a starting and mean value of 0.0125 and 0.0091, and standard deviation 0.02 and 0.015, for the COI and 16S rates, respectively. We assign non-informative uniform priors for the nuclear genes, with lower and upper values 0.0001 and 0.02, respectively. We ran three independent chains of 50 million generations each, sampling every 10,000 generations. We monitored the chain convergence, the correct mixing, and the number of generations to discard as burn-in (10%) with Tracer v. 1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). We used the accompanying programs Logcombiner and Treeannotator to combine the chains and infer the tree with maximum clade credibility. Pairwise patristic distances between target species pairs using the calibrated branch lengths were estimated with the R package APE (Paradis, Claude, & Strimmer, 2004).

Distribution range

We considered the geographic distance as a proxy of the potential competition (Morales-Castilla, Matias, Gravel, & Araújo, 2015). This stems from the idea that spatial proximity enhances a probability that a members of two species encounter each other and that some interaction between them unfolds (Delić, Trontelj, Zakšek, & Fišer, 2016a). We assembled a database of occurrence localities based on available literature data (Mammola et al., 2019) updated with few recently discovered additional localities (Figure 1a). To represent the geographic distance between species pairs, we calculated the distance between the centroids of the distribution of

each species. To take into account the fact that *Troglohyphantes* in this area do not occur at low altitudes (Isaia et al., 2010), we calculated centroid distances as a cost weighted distance. We used least-cost path analysis in the R package *gdistance* (Jacob, 2018), with an altitudinal transition raster with a high cost weight applied to cells below 500 m a.s.l. As a further measure of range overlap we followed Mammola et al. (2019), expressing each species' distribution range as the elevational range extent, namely the maximum and minimum elevations across all species occurrence records. We estimated range overlap as the pairwise overlaps among the elevational range extents calculated for each species. As in the case of co-occurrence matrixes, we interpreted the range overlap as a proxy for the potential competition among species (Fišer, Luštrik, Sarbu, Flot, & Trontelj, 2015).

Statistical analyses

Functional space characterization

We studied morphological similarity among *Troglohyphantes* species by analysing variations in their functional traits in a multidimensional morphospace (Blonder, 2019). To delineate the geometry of the hyperspace, we constructed n -dimensional hypervolumes for all species using a Gaussian kernel density estimator (Blonder, Lamanna, Violle, & Enquist, 2014). To minimize collinearity among traits and incorporate in the hypervolume estimation the categorical variable "pigmentation", we used the distance-based approach by Laliberté & Legendre (2010) and its generalization to the case of kernel n -dimensional hypervolumes proposed by Carvalho & Cardoso (2018). In a first step, we applied a Gower dissimilarity measure (Gower, 1971) to the complete trait matrix. Then, we analysed the resulting distance matrix through Principal Coordinate Analysis (PCoA) in order to extract orthogonal morphological axes for hypervolume construction. We retained the first three PCoA axes to delineate hypervolumes with a Gaussian kernel density estimator (Blonder et al., 2018). We automated the choice of bandwidth for each axis using a cross-validation estimator (Duong & Hazelton, 2005), which is computationally slower but nonetheless has lower predictive error rates than the default Silverman estimator (Blonder et al., 2018). To compare the functional space of the 13 species, we constructed the triangular matrixes of pairwise niche centroids and minimum

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functional distances between their n -dimensional hypervolumes. We expressed functional similarity via distance metrics owing to the fact that most generated hypervolumes were largely disjunct (Mammola, 2019a).

Testing the predictions of this study

We applied null modelling to test whether species occupying a similar microhabitat have a higher overlap in their functional niche, as well as a higher phylogenetic and geographic distance, than expected from a random sorting of species. We expressed the observed value as the average of the distance values among the species inhabiting a given habitat. Then, we repeatedly (999 times) randomly subsampled k species from the species pool, where k equals the number of species within a given microhabitat, and extracted their mean distance values for each permutation. Permutation was constrained so that for any given microhabitat, one of the original species was kept, and the remaining were randomly shuffled. The null hypothesis of random sorting of species was rejected if the observed value was higher than the 97.5 percentile or lower than the 2.5 percentile of the 999 randomizations. For each permutation, we estimated the Standard Effect Size (SES) and p -value.

To estimate the relative contribution of functional trait divergence, phylogenetic relatedness, and geographic distance—a surrogate of interspecific competition—in determining the observed pattern of microhabitat specialisation, we relied on variance partitioning analysis (Borcard, Legendre, & Drapeau, 1992). From the distance and overlap matrices of the 13 species, we extracted the values of functional, phylogenetic and geographic distance between any two pairs of species. In parallel, we created a dummy variable (0–1, discrete) by assigning a value of one to each comparison between any two species occurring within the same microhabitat, and a value of zero between any two species from different microhabitats. Using this dataset, we modelled the contribution of functional, phylogenetic, and geographical distance between pairs of species from the same habitat (value of 1 in the dummy variable) versus different microhabitat (value of 0 in the dummy variable), using Bernoulli generalized linear models (GLMs). Prior to model fitting, we scaled all variables to facilitate model convergence. We assessed the variance explained by each model using pseudo R^2 . In turn, we used pseudo R^2

values to evaluate the contribution of each variable and combination of variables by partitioning their explanatory power using the 'modEvA' R package (Barbosa, Brown, Jimenez-Valverde, & Real, 2015).

Finally, we explored the extent to which morphological and phylogenetic differentiation occurs among coexisting species via generalized linear mixed model (GLMMs), modelling the functional distance and the phylogenetic relatedness as a function of the range overlap. The species identity was included as a random factor to account for data dependence. Considering the nature of the data, a gamma distribution with a log link function was used in all GLMMs. Prior to model construction, we scaled variables to facilitate model convergence and log-transformed the altitudinal range overlap to homogenize its distribution. For this analysis, we excluded comparison between species pairs belonging to the same microhabitat, owing to the fact that these never coexist (Deeleman-Reinhold, 1978; Isaia et al., 2017; Mammola, Arnedo, et al., 2018).

RESULTS

Western alpine *Troglohyphantes* spider species considered in this study showed substantial variability in morphological traits, especially those associated with subterranean adaptation. There was a clear functional signal in most of the traits considered (Figure 2). Species adapted to cave entrance habitats were in general pigmented, with regular development of eyes (Figure 1b), and larger in size (Figure 1c). Species from shallow cave habitats were both small- and large-sized species (Figure 1c), all showing an intermediate level of regression of the ocular area (Figure 1b) and a slight shortening of the length of cephalothorax profile (Figure 1d). Deep cave habitat species had the most pronounced cephalothorax profile reduction (Figure 1d; but see greater variability in *T. pedemontanus*) and eye regression (Figure 1b). Finally, MSS-adapted species were the smallest in size (Figure 1c), showed moderate regression of eyes (Figure 1b), but no substantial profile reduction (Figure 1d).

Phylogenetic analyses resolved the Western alpine *Troglohyphantes* of interest into four clades (Figure 3). Three of the clades were exclusive in terms of the cave microhabitat occupied by the species included. Interestingly a fourth clade was formed by species from different cave microhabitats. The species *T. iulianae* was not found to be closely related to any of the four clades. With exception of species living in cave entrances, each subterranean habitat was colonized twice independently (Figure 3).

Using the full matrix of functional morphological traits, we successfully generated gaussian kernel 3-dimensional functional hypervolumes for all the species considered in the study (Figure 4). Intersection between hypervolumes of any two pairs of species from different microhabitats was zero in most cases and always below 0.01, indicating that hypervolumes were *de facto* fully disjunct. Volume of the 3-dimensional hypervolumes of the different species varied by one order of magnitude (Table 1), with *T. pluto* having the most voluminous multidimensional space (6.4567) and *T. lanai* the least voluminous one (0.5532). There was no significant difference between the volumes of the species' hypervolumes depending on the habitat occupied (ANOVA: $F_{1,11} = 0.90$, $p = 0.36$).

Species adapted to the same microhabitat clustered together with respect to at least two PCoA orthogonal axes (Figure 4b) and were well segregated based on

their microhabitat specialisation when looking at their functional space in 3-dimensions (Figure 4a). Null modelling further showed that species belonging to the same microhabitat were morphologically more similar than expected from a random sorting of species among habitats (Figure 5a). This was true for the two functional distance metrics considered (distance between centroids: $SES = -2.35$; $p < 0.01$; minimum distance: $SES = -1.74$; $p = 0.03$). Accordingly, in all subsequent analyses we arbitrarily selected the distance between hypervolume centroids as measure of functional distance. The mean genetic distance among species from the same habitat was lower than expected from a random sorting of species among habitats ($SES = -3.20$; $p < 0.01$; Figure 5b). Conversely, geographic distance between species was not significantly lower for species belonging to the same habitat ($SES = 0.45$; $p = 0.63$; Figure 5c). The same, not significant result, was obtained when using species range overlap rather than geographic distance in the null modelling ($SES = 0.49$; $p = 0.59$).

According to the variance partitioning analysis, we observed a substantial difference in the contribution of functional morphology, phylogeny, and geography in determining microhabitat occupation (Figure 5d). The joint effect of functional and phylogenetic distance explained over 36% of the variance explained by the model, whereas functional distance alone accounted for an additional ~17%. The contribution of geography was small, whether alone (pseudo $R^2 = 0.02$) or in conjunction with functional or phylogenetic distance (pseudo $R^2 = 0.02$). Phylogenetic distance accounted for an additional 10% of variance, while over thirty percent of variance in the dataset remained unexplained.

Finally, regression analyses indicated that as the range overlap between species from different habitats increases, their functional and genetic distance significantly increase (Morphology: Estimated $\beta \pm S.E. = 0.26 \pm 0.04$, $p < 0.01$; Genetic: 0.10 ± 0.02 , $p < 0.01$), a result suggesting that species are more often prone to coexist when their morphological and genetic dissimilarity is high (Figure 5e, 5f).

DISCUSSION

Our results point out that ecological specialisation in subterranean microhabitats emerged through both historical contingency and environmental filtering. Conversely,

the role of interspecific competition remained inconclusive. All microhabitat types, with exception of cave entrances, were independently colonized twice and therein, *Troglohyphantes* species independently attained non-random similarity in morphological functional traits. This is a typical evolutionary pattern observed in ecological settings characterized by stringent environmental constraints, like deserts (Melville, Harmon, & Losos, 2006), sulfidic springs (Tobler et al., 2015), or deep oceans (Sumner-Rooney, 2018). At least three *Troglohyphantes* lineages colonized the Western Alps subterranean environment, each of them subsequently adapting to different subterranean microhabitats (Figure 3). These results are reflected in variance partitioning analysis, where most of the variation explains the joint effect of phylogenetic origin and functional traits (environmental filtering). Yet, the range overlap corresponds to degree of between-species divergence, whether phylogenetic or in functional traits, and might imply that competitive interactions contributed to diversification and/or filtering.

Our results partially support previous findings related to diversification of subterranean animals, but also unveil new aspects worth to be further discussed. Traditionally, morphological variation in subterranean species had been ascribed to time since colonization, i.e., the oldest species are the most adapted (Poulson, 1963). This hypothesis received only little support (Derkarabetian et al., 2010), but can be rejected in this study since the crown age of the clade of species living in cave entrances [$((T. lucifer + T. lucifuga) + T. pluto)$; mean age estimation (confidence interval) = 7.5 (16–3) My] is not younger than the clade of the most specialised eyeless species from deep caves [$((T. bolognai + T. bolognai) + T. konradi) + T. pedemontanus$]; 6 (12–2.5) My] (Figure 3). By contrast, several studies on subterranean beetles (Martins & Ferreira, 2019; Vergnon et al., 2013), amphipods from caves (Delić, Trontelj, Zakšek, & Fišer, 2016b; Fišer et al., 2015; Trontelj et al., 2012; Zakšek, Delić, Fišer, Jalžić, & Trontelj, 2019), interstitial habitats (Fišer, Delić, Luštrik, Zagmajster, & Altermatt, 2019) or deep wells (Hutchins, Schwartz, & Nowlin, 2014), but also spiders (Arnedo, Oromí, Múrria, Macías-Hernández, & Ribera, 2007; Mammola, Arnedo, et al., 2018; Mammola et al., 2019), suggested that variation in functional traits corresponds to ecological diversification of subterranean species. These studies strongly concur with the results presented here and support the hypothesis that rather than evolutionary dead ends,

subterranean organisms continue evolving and actively specialising into new microhabitats (Cieslak, Fresneda, & Ribera, 2014; Copilaş-Ciocianu, Fišer, Borza, & Petrusek, 2018; Stern et al., 2017).

The role of phylogeny in ecological specialisation of subterranean species, on the other hand, has been incompletely explored. Few studies have used phylogenies to show the convergent origin of functional traits (Fišer et al., 2019; Trontelj et al., 2012), but none have explored the role of historical contingency in subterranean community assembly. Results of our study imply that common phylogenetic origin explains ecological diversity of subterranean species as importantly as convergent evolution. The potential importance of historical contingency might be exemplified by morphologically similar species, commonly found in all hitherto studied subterranean species (Delić, Trontelj, Rendoš, & Fišer, 2017; Esposito et al., 2015; Hedin, 2015; Niemiller, Near, & Fitzpatrick, 2012). Clades of these so-called cryptic species might share ecological niches as a result of historical contingency, a hypothesis that should be explored further (Fišer, Robinson, & Malard, 2018). From a broader perspective it should not pass unnoticed that phylogenetic niche conservatism explains substantial variation on a relatively small geographic scale. It has been suggested that this phenomenon applies particularly well to large-scale biogeographic patterns (Wiens, 2008). Insofar as subterranean spiders are generally poorly mobile and with small ranges (Hedin, 1997; Mammola et al., 2015; Snowman, Zigler, & Hedin, 2010), a possible explanation is that the size of the studied region was large enough to detect the effects of historical contingency.

Finally, the role of competition remains elusive. Statistic tests did not yield significance to the hypothesis that species from the same microhabitats are evenly dispersed in space (i.e. unexpectedly large distances between pairs of species; Figure 5c) and explains only small fraction of ecological variation (Figure 5d). The explanation for this result could be found in the isolated nature of cave systems (Chiari et al., 2012; Rizzo, Sánchez-Fernández, Alonso, Pastor, & Ribera, 2017) and the limited dispersal ability of cave-dwelling spiders. Based upon these specificities, we could argue that the likelihood of interspecific competition in any pair of cave-dwelling species geographically separated is effectively zero. However, this result might also be an artifact caused by the uneven range sizes and species distribution (Figure 1a) which might inflate the null model. Additionally, using the extent of

overlap in their distribution range as a surrogate for the degree of coexistence between two species might not be precise enough to capture true interspecific interactions. Given that species from the same microhabitats are strictly allopatric, we suggest that competition operates at the microhabitat level. The latter idea is supported also by results of generalized linear mixed models (Figure 5d, e) implying that interspecific competition relaxes with degree of ecological and phylogenetic divergence. This finding provides a statistical confirmation to the empirical observation by Deeleman-Reinhold (1978) that coexistence in *Troglohyphantes* is most likely to occur between species that belong to different species-complexes (*sensu* Isaia et al., 2017). In fact, in the only known case of two sister species of *Troglohyphantes* coexisting at the local scale, the species' morphology diverged substantially to enhance the occupation of distinct microhabitats (cave *versus* MSS; Mammola, Arnedo, et al., 2018). This result is also partially congruent to similar analysis of interstitial amphipods, where the frequency of co-occurrence records showed a positive relationship with degree of their ecological differentiation, but was not affected by phylogenetic relatedness (Fišer et al., 2019).

More generally, it is worth noting that the role of interspecific competition in driving subterranean community assembly remains debated (Mammola, 2019b). Some studies indicate that interspecific competition is an important force structuring subterranean communities (Culver, 1976; Delić et al., 2016b; Eberly, 1960; Fišer et al., 2015; Vergnon et al., 2013), whereas other imply that ecologically divergent species may assemble into communities avoiding competition (Zakšek et al., 2019). Overall, this is a radically different pattern from the one typically documented in highly dispersive species, such as birds (Baselga, Gómez-Rodríguez, & Lobo, 2012; Herrera-Alsina & Villegas-Patraca, 2014) or bats (Schoeman, Goodman, Ramasindrazana, & Koubínová, 2015). In tropical lowland hummingbird communities, for instance, biotic interactions appear to be even more important than habitat filtering in driving community assembly processes (Graham, Parra, Rahbek, & McGuire, 2009).

The specialisation of *Troglohyphantes* to specific microhabitats within caves and the morphological differentiation thereof is apparently enabling species pairs to coexist at the community level, i.e. within a single cave or subterranean system. This morphological differentiation, primarily driven by environmental filtering and only

secondarily by interspecific competition, led to the establishment of replicates of equivalent subterranean communities across the Alps. This might provide an explanation for the diversification undergone by this genus in the subterranean environment, which currently includes 134 nominal species (World Spider Catalog, 2020), overall accounting for almost a quarter of the total subterranean spider diversity in Europe (Mammola, Cardoso, et al., 2018). Similar replicated community assembly patterns have been classically documented in oceanic islands (Case, 1983; Gillespie, 2004; Lockwood, Moulton, & Anderson, 1993; Losos & Ricklefs, 2009; Macías-Hernández, Oromí, & Arnedo, 2008) and in mountain summit communities (Schöb, Butterfield, & Pugnaire, 2012; Tanentzap et al., 2015). Our results suggest that playing the evolutionary tape twice (Blount, Lenski, & Losos, 2018) also resulted in the same community assembly patterns across other island-like habitats (*sensu* Itescu 2019).

Data Availability statement

Data supporting this study and R code for replicating the analyses are available in Dryads: <https://doi.org/10.5061/dryad.sn02v6x15> (Mammola et al., 2020). DNA sequence are deposited in GenBank® (see accession codes in Appendix S1). All studied specimens are stored in 75% ethanol either at the Department of Life Sciences and Systems Biology, University of Turin (Marco Isaia's collection) or at the Museo di Scienze Naturali "E. Caffi" of Bergamo (Italy).

Supplementary materials

Appendix S1. List of specimens sequenced in this study with voucher information, DNA code, and GenBank® access code.

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TABLES

Table 1. Species of *Troglohyphantes* considered in this study. Adaptation= Degree of morphological adaptation to the subterranean medium based on Mammola et al. (2019). In parentheses, species not included in the initial morphological clustering, but assigned *a posteriori*; Habitat= the habitat occupied by each species (see section “Microhabitat classification” for more details); N= the number of specimens measured. Hypervolume= estimated volume of the 3-dimensional functional hypervolume of each species—values are multiplied by 1000 to increase resolution.

Species	Adaptation	Microhabitat	N	Hypervolume
<i>Troglohyphantes</i> sp_1, under description	(intermediate)	Shallow cave	8	0.9482
<i>Troglohyphantes</i> sp_2, under description	(intermediate)	Shallow cave	–	–
<i>T. bolognai</i> Brignoli 1975	high	Deep cave	11	0.4014
<i>T. bornensis</i> Isaia & Pantini 2008	intermediate	Shallow cave	10	3.5070
<i>T. giachinoi</i> Isaia & Mammola 2018	(intermediate)	MSS	8	2.2007
<i>T. iulianae</i> Brignoli 1971	intermediate	MSS	9	5.0987
<i>T. konradi</i> Brignoli 1975	high	Deep cave	10	3.1421
<i>T. lanai</i> Isaia & Pantini 2010	high	Deep cave	11	0.5532
<i>T. lucifer</i> Isaia et al. 2017	low	Cave Entrance	11	2.1620
<i>T. lucifuga</i> (Simon, 1884)	low	Cave Entrance	11	1.9071
<i>T. nigraerosae</i> Brignoli 1971	intermediate	Shallow cave	9	2.5847
<i>T. pedemontanus</i> (Gozo, 1908)	high	Deep cave	12	3.8537
<i>T. pluto</i> Caporiacco 1938	low	Cave Entrance	13	6.4567
<i>T. vignai</i> Brignoli 1971	intermediate	Shallow cave	9	1.4852

Table 2. Morphological traits considered in the analyses, with hypotheses on their functional meaning.

Trait	Variable construction	Functional meaning
Leg I–V length	Length of leg I–V (sum of individual leg articles)	Leg length is a <i>proxy</i> for overall body size (Elgar, Ghaffar, & Read, 1990). In subterranean spiders, leg length is often related with habitat (pore) size (Mammola & Isaia, 2017). In <i>Troglohyphantes</i> , leg elongation preferentially occurs in subterranean species (Deeleman-Reinhold, 1978)
Sternum ratio	Ratio between sternum maximum length and width	A <i>proxy</i> for overall body size (Hagstrum, 1971). In subterranean species, size is possibly related to habitat (pore) size (Pipan & Culver, 2017)□
Cephalothorax height/length	Ratio between height and length of the cephalothorax	In <i>Troglohyphantes</i> , the flattening of the cephalothorax is meant to occur with increasing levels of subterranean adaptation (Isaia & Pantini, 2010)□
Anterior eyes regression	Sum of AME and ALE diameters, divided by the total length of the anterior line	In spiders, eye regression is the most evident morphological change to the subterranean conditions (Mammola & Isaia, 2017). In <i>Troglohyphantes</i> , the anterior median eyes are the first undergoing regression (Deeleman-Reinhold, 1978)□
Posterior eyes regression	Sum of PME and PLE diameters, divided by the total length of the eye region	
Total eyes regression	Anterior eyes + Posterior eye	
Pigmentation	Categorical variable reflecting presence/absence of abdomen pigment.	In spiders, with the adaptation to the subterranean conditions, body pigment is generally lost (Mammola & Isaia, 2017)□
Fang	Length of the fang	The dimension of fangs provides information on dietary requirements and trophic specialization

FIGURE CAPTIONS

Figure 1. *Troglohyphantes* distribution in the Western Alps and Northern Apennine (**a**), and variations in some of their main morphological features (**b–d**). **a)** For each species, the individual localities and the centroids of the distribution are reported. For species occurring in more than 5 localities, the Minimum Convex Polygon (MCP) encompassing all localities is also drawn for visual presentation. Shades of grey in the background represent altitude. **b–d)** Boxplots are coloured according to the occupied microhabitats. Some species names are abbreviated. See Table 2 for an explanation of the functional meaning of traits.

Figure 2. Variations in the habitus and key morphological traits of Western alpine *Troglohyphantes* adapted to different subterranean microhabitats. See Table 2 for an explanation of the functional meaning of traits. Photos by Francesco Tomasinelli and Emanuele Biggi. Original drawing by Elena Pelizzoli (modified).

Figure 3. Chronogram corresponding to the maximum clade credibility tree as inferred in BEAST analysis. Circles on internal nodes denote support values (PP: Bayesian posterior probability; BS: maximum likelihood bootstrap). Colour coding: black= PP > 95% and BS >75; grey= clades recovered with support values below the former threshold; white= clades not recovered. The tree was rooted using *Troglohyphantes oromii* (Ribera & Blasco, 1986) and *T. roquensis* Barrientos & Fernández-Pérez, 2018 from the Canary islands. The position of *T. giachinoi* is inferred from COI data only (Mammola, Arnedo, et al., 2018).

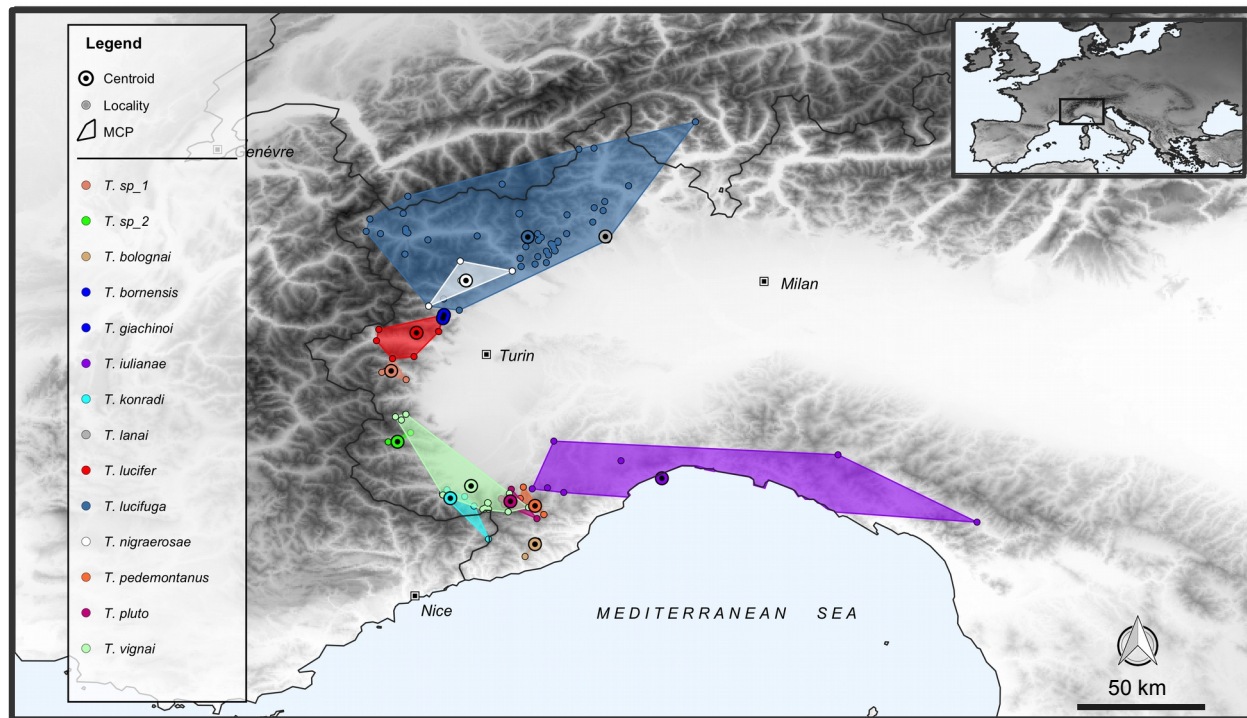
Figure 4. Estimated 3-dimensional hypervolume for Western alpine *Troglohyphantes*, based on the first three axes of the Principal Coordinate Analysis (PCoA) performed on the Gower dissimilarity matrix of all morphological traits. Random points are coloured based on the habitat occupied. **a)** Three-dimensional representation of the morphological space of *Troglohyphantes* spiders. **b)** Bi-dimensional representation of the morphological space. Contour lines delimiting random points (niche boundary) are drawn for visual presentation.

Figure 5. Contribution of functional, genetic and geographic distance in driving habitat specialization in Western alpine species of *Troglohyphantes*. **a–c)** Distribution of expected values versus mean observed value of (**a**) distance between hypervolume centroids, (**b**) phylogenetic distance and (**c**) distance between distribution centroids of Western alpine *Troglohyphantes*, based on null modelling analysis. The observed value is set at the mean values among all the species of given habitat, whereas expected distribution is obtained by 999 randomly subsampled of k species from the species pool, where k equals the number of species within a given habitat, and extracting their mean values for each permutation. **d)** Venn diagram showing the contribution of each variable and combination of

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variables in driving habitat differentiation, based on the variance partitioning analysis. Variable contribution is expressed as pseudo R^2 . **e)** Predicted positive relationship between functional distance and altitudinal range overlap, based on the result of gamma GLMM. **f)** Predicted positive relationship between genetic distance and altitudinal range overlap, based on the result of gamma GLMM. In **e** and **f**, black lines represent fitted relationships, whereas grey surfaces are 95% confidence intervals. Only fixed effects are shown.

(a)



Legend

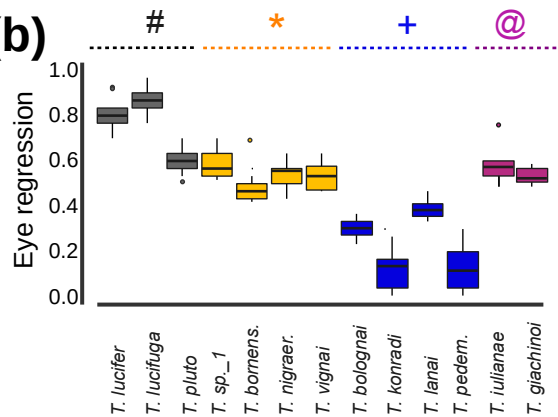
(b-d)

- + Deep cave habitats
- * Shallow cave habitats
- # Cave entrance/external
- @ MSS/interstitial

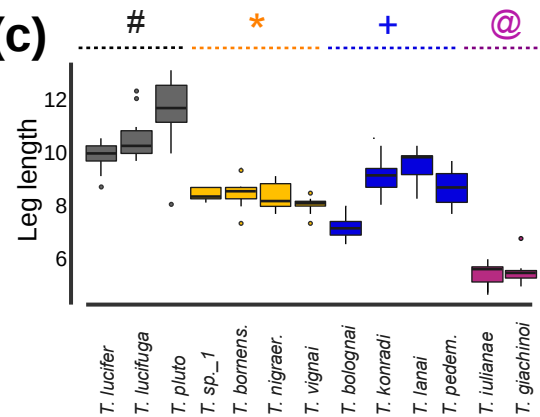
○ outlier

max
75%
median
25%
min

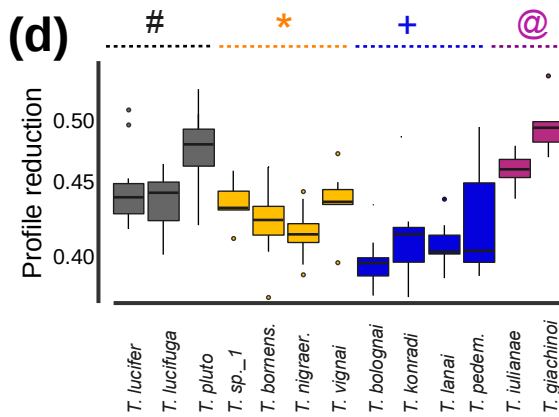
(b)



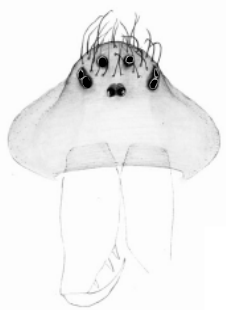







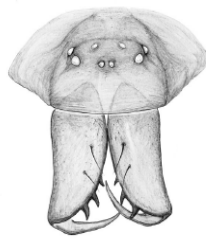


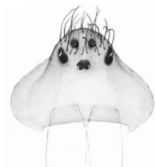




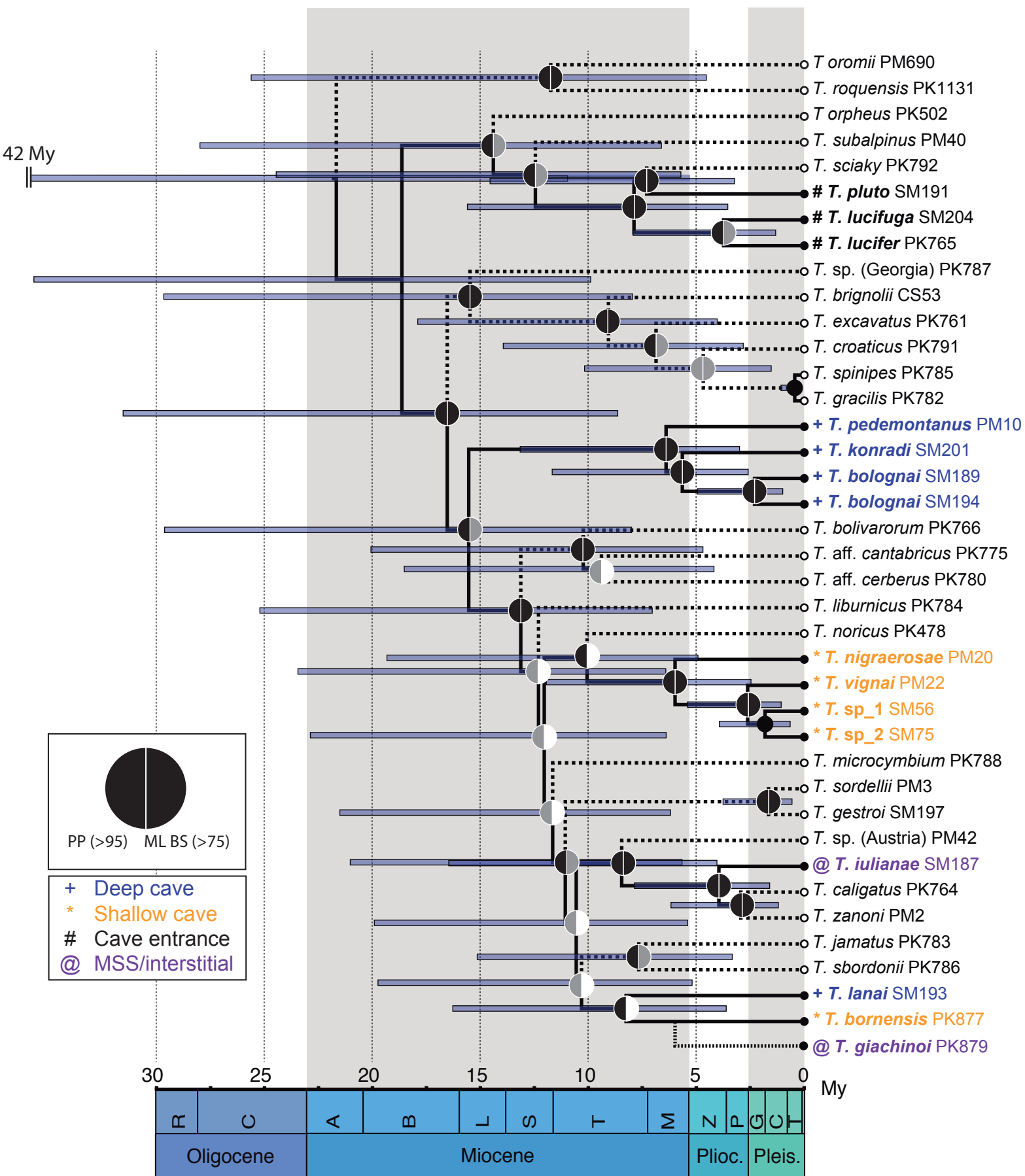
(c)



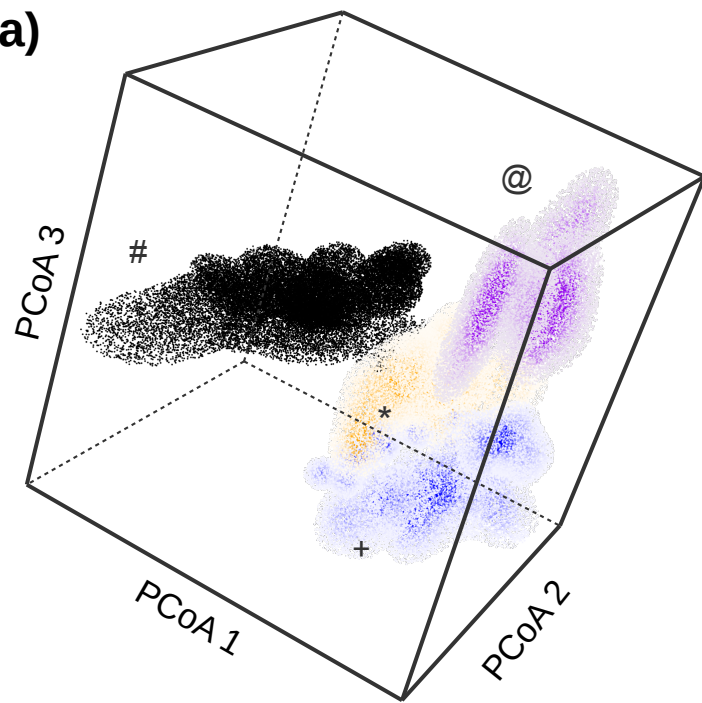
(d)



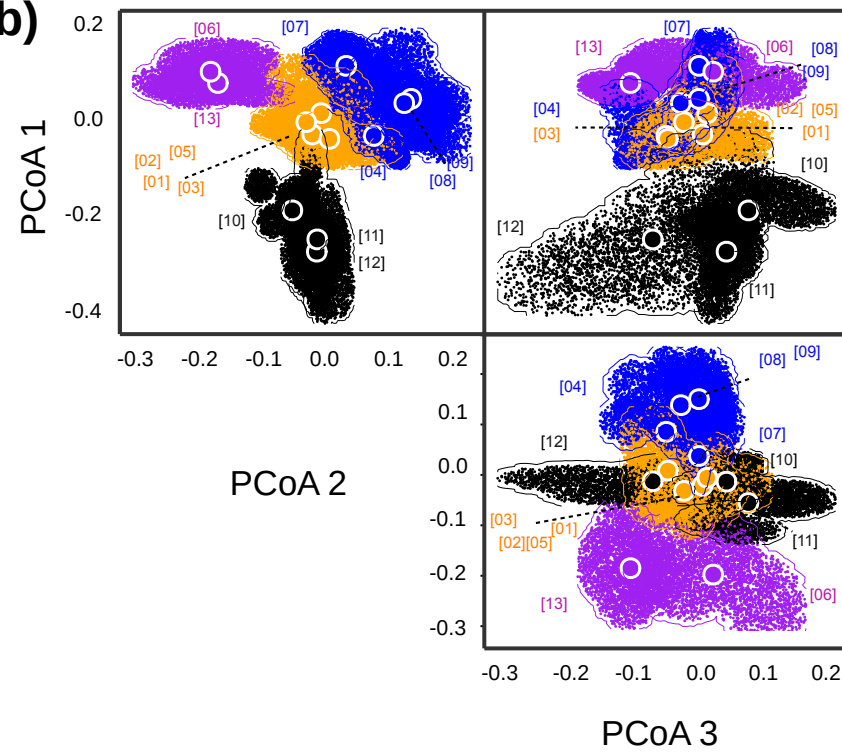
Habitus		Habitat Species	Eyes regression Profile reduction	Pigmentation	Size
 <p>(Photo: Francesco Tomasinelli)</p>		Cave entrance/external <i>T. lucifer</i> <i>T. lucifuga</i> <i>T. pluto</i>			
		Shallow cave habitats <i>T. bornensis</i> <i>T. nigraerosae</i> <i>T. vignai</i> <i>T. sp_nov 1</i>			
 <p>(Photo: Francesco Tomasinelli)</p>		Deep cave habitat <i>T. bolognai</i> <i>T. konradi</i> <i>T. lanai</i> <i>T. pedemontanus</i>			
		MSS/Interstitial <i>T. giachinoi</i> <i>T. iulianae</i>	 <p>1 mm</p>	 <p>1 mm</p>	 <p>1 mm</p>



(a)



(b)

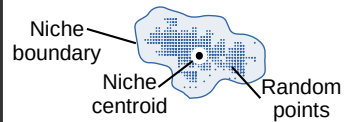


Legend

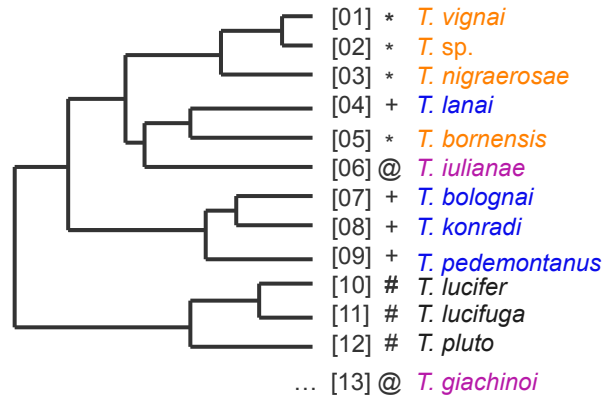
Microhabitat

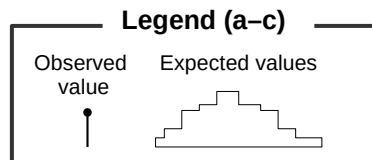
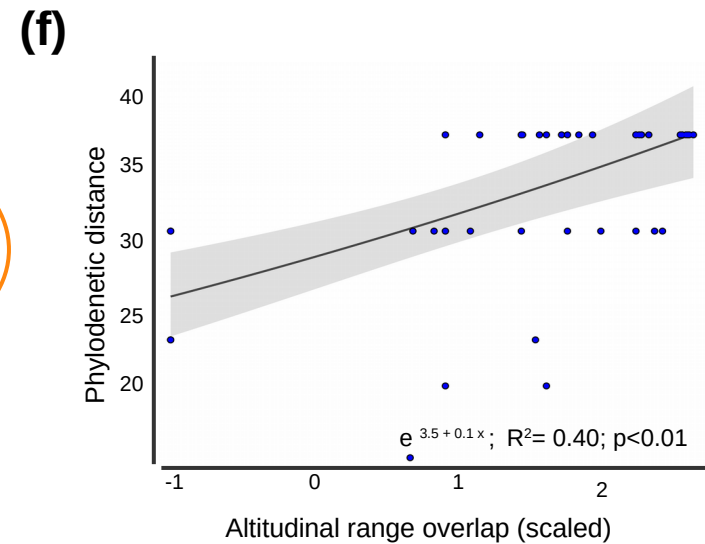
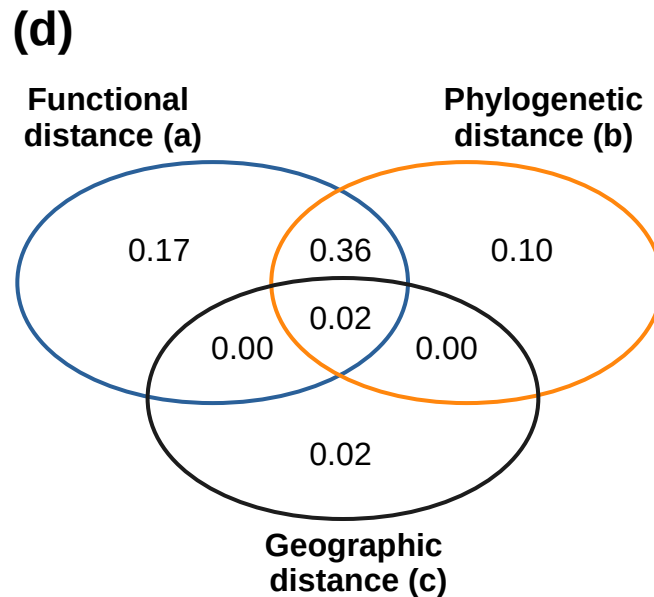
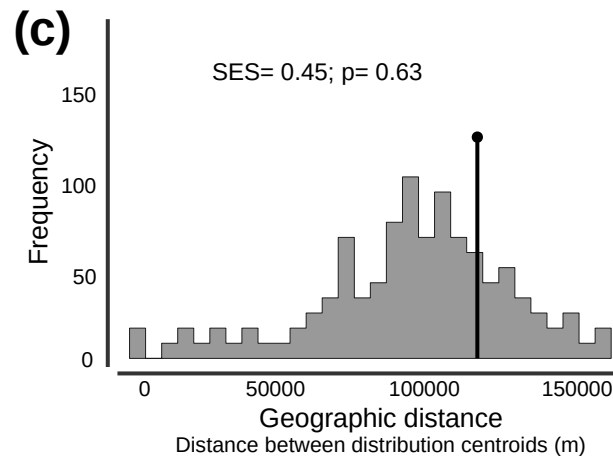
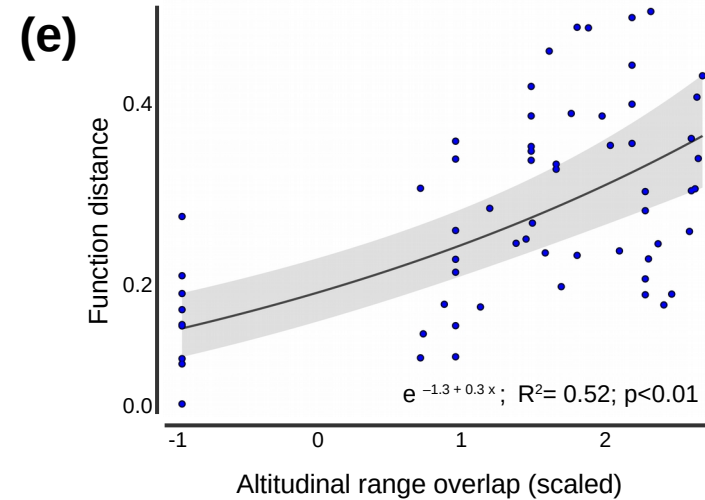
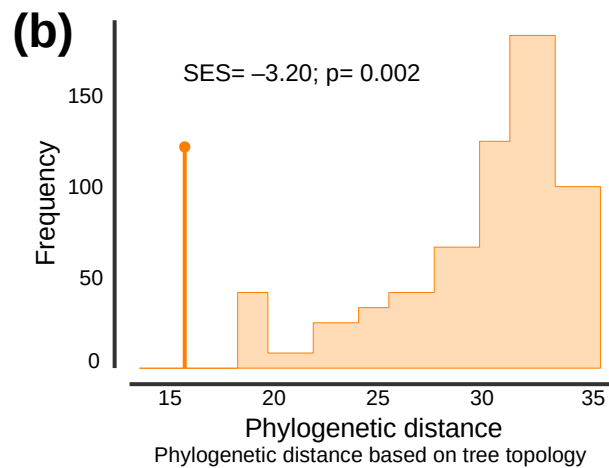
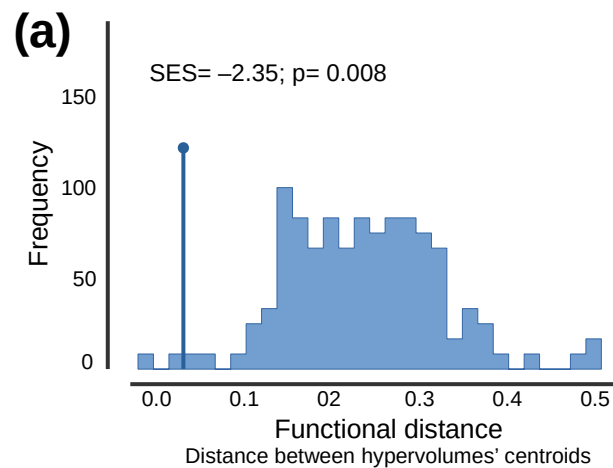
- + Deep cave habitats
- * Shallow cave habitats
- # Cave entrance/external
- @ MSS/interstitial

Hypervolume (a)



Pruned tree based on topology in Figure 3





Unexplained = 0.36